APPENDIX B

MUTAGENICITY AND GENOTOXICITY GUIDELINES

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The U.S. Environmental Protection Agency (EPA) has developed and published *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986). The information in this appendix was primarily taken from that document. Quantitative assessment of mutagenicity requires two steps: (1) determining the heritable effect per unit of exposure (dose-response) and the relationship between mutation rate and disease incidence, and (2) combining dose-response information with anticipated levels and patterns of human exposure in order to derive a quantitative assessment of risk (U.S. EPA, 1986). Current EPA guidance on mutagenicity risk assessment specifies that:

> Dose-response assessments can presently only be performed using data from in vivo, heritable mammalian germ-cell tests, until such time as other approaches can be demonstrated to have equivalent predictability. (U.S. EPA, 1986).

The relationship between in vitro assay results and effects in mammalian systems is not sufficiently characterized to be able to use in vitro assays as the basis for developing a dose-response assessment.

An example of the type of study that could be used for mutagenicity risk assessment is an assay that directly detects heritable health effects in the first-generation offspring. Human risk estimates are obtained by extrapolating the induced mutation frequency or observed phenotypic effect downward to the anticipated level of human exposure (EPA, 1986). No one extrapolation model has been identified as the most appropriate. The Agency notes that departures from linearity at low exposure and exposure rates has been observed for at least one chemical. According to EPA, “[t]he Agency will consider all relevant models for gene and chromosomal mutations in performing low-dose extrapolations and will choose the most appropriate model. This choice will be consistent with both the experimental data available and current knowledge of relevant mutational mechanisms” (U.S. EPA, 1986).

The factors that should be considered in evaluating chemicals for mutagenic activity include:

- Genetic endpoints
- Sensitivity and predictive value of the test systems for various classes of chemical compounds
- Number of different test systems used for detecting each genetic endpoint
- Consistency of the results obtained in different test systems and different species
- Aspects of the dose-response relationship
- Whether the tests are conducted in accordance with appropriate test protocols agreed upon by experts in the field (U.S. EPA, 1986).

Although there are often no in vivo data available on a chemical, there are in vitro assay results for most common chemicals. These data may be used qualitatively to evaluate the mutagenicity of chemicals. They are often used as supporting evidence in carcinogenicity, developmental toxicity, and reproductive toxicity evaluations.

Various types of test results may be obtained regarding mutagenicity. In evaluating interactions in the mammalian gonad, two possible types of evidence have been specified. Evidence for chemical interactions in the mammalian gonad may be considered sufficient if it is demonstrated that "an agent interacts with germ-cell DNA or other chromatin constituents, or that it induces endpoints such as unscheduled DNA synthesis, sister-chromatid exchange, or chromosomal aberrations in germinal cells" (U.S. EPA, 1986). Suggestive evidence of interaction in the mammalian gonad "includes effects such as sperm abnormalities following acute, subchronic or chronic toxicity testing, or finding of adverse reproductive effects such as decreased fertility, which are consistent with the chemical's interaction with germ cells" (U.S. EPA, 1986).

In practice, the outcomes of developmental and reproductivity toxicity testing often do not indicate the type of toxicity that leads to effects such as decreased fertility. The causes of decreased fertility range from mutagenicity leading to early fetal death or failure to implant to maternal, paternal, or fetal toxicity. Positive significant mutagenicity studies along with fetal toxicity or reduced fertility may be suggestive that mutagenic action was a causal action. Developmental toxicity has been studied in most chemicals (other than frank teratogens) relatively recently. The use of the data from these studies with other types of data such as mutagenic, pharmacokinetic, and reproductive system studies is developing; however, currently there is not clear guidance on these types of evaluations.

EPA has addressed the issue of weight-of-evidence for mutagenicity by providing a classification scheme with categories presented in decreasing order of strength of evidence:

1. Positive data derived from human germ-cell mutagenicity studies

2. Valid positive results from studies on heritable mutational events in mammalian germ cells

3. Valid positive results from mammalian germ-cell chromosome aberrations studies that do not include an intergenerational test

4. Sufficient evidence for a chemical's interaction.

Reference

U.S. EPA (Environmental Protection Agency). 1986. Guidelines for mutagenicity risk assessment. *Federal Register.* 51(185):34006-34012.